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Mapping of promoter sites utilized by T3 RNA polymerase on T3 DNA

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ABSTRACT

Promoter locations for the T3 RNA polymerase on the physical map of T3 DNA have been determined. Through the use of conditions favoring the synthesis of RNA from the class II region, an agarose-formaldehyde gel system which improves the resolution of high molecular weight RNAs, and template DNA that was cut by one of several restriction endonucleases prior to transcription, seventeen promoter locations for the T3 RNA polymerase have been mapped. Ten promoters have been identified in the class II region and one promoter has been identified in the early (class I) region. The locations of previously mapped class III promoters and the internal termination signal for the T3 RNA polymerase have been mapped more precisely than in previous reports. The resulting transcription map demonstrates a striking similarity to the transcription map of bacteriophage T7.

INTRODUCTION

Bacteriophage T3 is one of a class of closely related phages which exhibit morphological, genetic and physiological similarities to bacteriophage T7 (1,2,3). During infection the genome of T3 [which consists of 38.7 kb of linear double-stranded DNA (4,5)] is transcribed by two RNA polymerases. Whereas the leftmost 20% of the DNA molecule is transcribed by the E. coli RNA polymerase, the remaining 80% is transcribed by a phage-specified RNA polymerase (the product of gene 1) (6,7,8).

In the related phage T7, expression of the genome occurs in three temporal classes (9,10). Class I genes are transcribed by the host RNA polymerase and are expressed early after infection. Class II genes, which are involved in DNA metabolism, are transcribed by the phage RNA polymerase from 4 until 16 minutes after infection. The genes of the class III region are involved in phage structure and assembly and are transcribed by the phage RNA polymerase from 8 minutes after infection until lysis. Because T3 genes of similar function are aligned in a fashion similar to those of T7 (11), we have maintained the convention of organizing the T3 genome into the corresponding regions (see Figure 1). However, the time course of transcription from these regions has not yet been investigated.

The T3 RNA polymerase has been purified and found to give highly selective transcription (6,12). Both *in vivo* and *in vitro* only one strand of the DNA is transcribed; the direction of transcription is from left to right relative to the genetic map (7,13,14). Using an agarose-acrylamide composite gel system, previous investigators were able to resolve six to eight major RNA species that are synthesized by the T3 RNA polymerase *in vitro* (15,16); these RNAs were mapped to the class III region of the genome (14). No specific RNAs originating from the class II region were identified. A similar situation existed in the bacteriophage T7 system but work in our laboratory has shown that transcription of the class II region of T7 DNA *in vitro* is enhanced in the presence of spermidine and low ionic strength and, furthermore, that the use of low percentage agarose gels and denaturing agents such as formaldehyde permits the resolution of the high molecular weight transcripts that arise from the class II region (17). Through the use of this technology we have been able to resolve additional late T3 RNA species not previously identified. By digesting the template DNA with one of several different restriction endonucleases prior to transcription and analyzing the size of the RNA products, we have mapped a total of seventeen promoters on the physical map of T3 DNA. Many of these promoters are in the class II region.

MATERIALS AND METHODS

Bacterial and phage strains. *E. coli* B and T3 Hausmann (3) were from the laboratory of E.K.F. Bautz. *E. coli* BL15 [an RNAse I$^-$ derivative of *E. coli* K12 (18)] was obtained from F.W. Studier. Because T3 does not adsorb well to K12 strains (3), a spontaneous host range mutant of T3 (T3hr2) capable of infecting *E. coli* BL15 was isolated and utilized for the purification of T3 RNA polymerase from T3hr2-infected BL15 cells. DNA from this mutant has the same Hpa I restriction pattern as T3 Hausmann.

Preparation of template DNA. Unlabeled T3 DNA was isolated from CsCl-purified viruses (19). DNA was digested with one of the following enzymes: Hind III, Bgl II, Bst E II, Xba I, Hpa I, or Mbo I, using conditions of cleavage recommended by the supplier (Bethesda Research Laboratories, Rockville, Md. and New England Biolabs, Beverly, Ma.). The DNA was phenol extracted, precipitated with ethanol, dried *in vacuo* and resuspended in TE buffer (10 mM Tris-HCl, pH 7.9; 1 mM EDTA-Na_4).

Purification of T3-specific RNA polymerase. *E. coli* BL15 was grown at 25°C to a density of $\sim 10^9$ cells/ml in 40 liters of complete M9 TB

medium (3) and infected with T3hr2 at a multiplicity of 5-10. Thirty minutes after infection the cells were poured over an equal volume of frozen buffer (0.01 M Tris-HCl, pH 7.4; 0.01 M $MgCl_2$) and collected by continuous flow centrifugation in a Sorvall centrifuge. The cells were stored at -20°C.

T3 RNA polymerase was purified from infected cells according to the protocol described by Kassavetis and Chamberlin for the purification of the T7 RNA polymerase through the ammonium sulfate precipitation step (20). The $(NH_4)_2SO_4$ precipitate was dissolved in buffer A (10 mM Tris-HCl, pH 8.0; 0.1 mM dithiothreitol (DTT); 5% glyerol) and applied to a 0.9 cm x 8 cm heparin-sepharose column (21). The column was washed with five volumes of buffer B (10 mM Tris-HCl, pH 8.0; 0.1 mM DTT; 10% glycerol; 0.05 M KCl) and eluted with one volume of buffer B containing 0.15 M KCl. The peak fractions of RNA polymerase were applied to a 0.8 cm x 10 cm phosphocellulose column which was washed with 5 volumes of buffer B containing 0.2 M KCl. The RNA polymerase was eluted with buffer B containing 0.6 M KCl. The peak fractions were dialyzed against storage buffer (40 mM Tris-HCl, pH 7.9; 0.2 mM DTT; 10 mM $MgCl_2$; 50% glycerol; 1 µM $ZnCl_2$) and stored at -20°C.

T3 RNA polymerase activity was assayed as described below using ^{3}H-UTP in the reaction mixture. Aliquots were spotted on filter paper discs, washed 3 times in 10% TCA, once in acetone, dried and counted in a toluene based scintillation fluid.

Synthesis of RNA and analysis by gel electrophoresis. ^{32}P-labeled RNA transcripts were prepared in 50 µl reactions containing 40 mM Tris-HCl, pH 7.9; 8 mM $MgCl_2$; 5 mM DTT; 4 mM spermidine-HCl; 5% (v/v) glycerol, 0.4 mM ATP, GTP, CTP; 0.1 mM ^{32}P-UTP (specific activity of 160 µCi/µmole); and 2.5 µg T3 DNA. The reaction mixtures were preincubated for 5 minutes at 37°C, whereupon 10 units (22) of T3 RNA polymerase were added. After incubation for 10 minutes, unlabeled UTP was added to a concentration of 1 mM and incubation was continued for an additional 3 minutes. Reactions were stopped by the addition of an equal volume of stop buffer [2% (w/v) SDS; 50 mM EDTA-Na_4; 100 µg/ml stripped yeast RNA]. RNA was precipitated by the addition of 2.5 volumes of ethanol, collected by centrifugation, and the pellet was dried *in vacuo*.

For gel analysis of high molecular weight RNA (>5x10^5 daltons), the RNA pellets were resuspended in 20 µl formaldehyde buffer (1.1 M formaldehyde; 0.1 M $NaPO_4$, pH 7.0; 0.1% SDS; 2 mM EDTA-Na_4) and sep-

arated by electrophoresis on an 0.8% agarose-formaldehyde gel (17,23) run for 6 hours at 55 V. For analysis of smaller transcripts, the pellet was taken up in 20 µl of cracking buffer and separated by electrophoresis in a 4% polyacrylamide gel (24) using a Tris-acetate buffer (31) for 2.5 hours at 100 V. The gels were dried and the RNA bands were visualized by autoradiography. The molecular weights of the RNAs were determined by comparison with late T7 transcripts run in the same gel (17).

Transfer of T3 RNA transcripts to nitrocellulose and hybridization to separated single strands of T3 DNA. Unlabeled T3 RNAs transcribed from a Bgl II-cut DNA template were separated by electrophoresis in a 0.8% agarose-formaldehyde gel for 6 hours at 55 V. The formaldehyde treated RNAs were transferred to nitrocellulose strips as follows (Brian Seed and David Goldberg, personal communication). The gel was soaked in 20X SSC for 45 min, and placed on top of two layers of Whatman No. 1 paper lying on top of foam rubber in a tray with 10X SSC. Uncovered paper was masked with parafilm. The gel surface was moistened with 10X SSC and covered with a nitrocellulose filter (Millipore, HAWP) moistened with 2X SSC. The nitrocellulose sheet was covered with 1 layer of Whatman No. 3 paper, a stack of paper towels and a glass plate. The blotting procedure was continued for 20 hours, after which the filters were air dried and baked in a vacuum oven at 70°C for 4 hrs.

Uniformly ^{32}P-labeled T3 DNA was prepared as previously described (5). The DNA was denatured and the individual strands were separated by agarose gel electrophoresis as described by Hayward (25). The bands were visualized by staining with ethidium bromide and ultraviolet illumination after electrophoresis (26), electroeluted into dialysis tubing (27), and precipitated in the presence of 0.3M sodium acetate, 10 mM magnesium acetate, 50 µg *E. coli* DNA, and 2.5 volumes of 95% ethanol. The DNA pellets were collected by centrifugation, washed once with 70% ethanol, dried *in vacuo* and resuspended in TE buffer.

The separated strands were incubated with nitrocellulose strips bearing the unlabeled T3 RNA transcripts in 1 ml containing 50% formamide; 5X SSC; 20 mM potassium phosphate, pH 6.5; 0.1% SDS; 1% (w/v) bovine serum albumin at 40°C for 14-18 hours. The filters were rinsed twice in wash buffer (2X SSC + 0.1% SDS) at room temperature for 15 min, once in wash buffer at 65°C for 15 min, once in wash buffer at room temperature for 5 min, and once in 1/2X SSC + 0.1% SDS at room temperature for 5 min. The filters were dried at room temperature and exposed to X-ray film.

RESULTS

Resolution of T3 RNA transcripts in agarose-formaldehyde gels.

When uncut T3 DNA is transcribed by the T3 RNA polymerase *in vitro* a complex pattern of transcription products is observed (Figure 2). The major class III transcription products are well resolved in the agarose-formaldehyde gel system; these RNA species are identified by Roman numerals [corresponding to a previous system of nomenclature (14,15)] and also by promoter location (see below). "Readthrough" products, which arise from the failure of the polymerase to consistently recognize the internal termination signal at 58.6%, are indicated by "-r" following the promoter position. Above the three prominent readthrough bands is an area of even higher molecular weight RNAs which are not resolved into discrete bands. These arise from initiation by the T3 RNA polymerase at promoters in the class II region (see below).

The enhanced production of class II transcription products under the reaction condtions that we have employed, and the increased resolution afforded by the agarose-formaldehyde gel system has necessitated the development of a new nomenclature to identify the large number of transcripts observed. We have adopted the convention of assigning each RNA band a number corresponding to the promoter location (in T3 units) which gives rise to that RNA species. As noted above, readthrough products are designated by the suffix "-r".

Analysis of RNA transcripts from Bgl II cut T3 DNA.

To identify and map class II promoter locations for the T3 RNA polymerase, T3 DNA was digested with one of several restriction endonucleases prior to transcription. Since all transcription of T3 DNA is from left to right (14, and see below), the effect of cutting the template with a restriction enzyme will be to truncate RNAs which are initiated to the left of the cut site and which ordinarily span the restriction site. RNAs that are initiated to the right of the restriction site will be unaffected by cutting of the template, as will those RNAs that are terminated before reaching the cut site. The disappearance of a particular band and the appearance of a corresponding band of lower molecular weight provide sufficient information to define the location of the promoter for each RNA species. Promoter locations determined in this manner are shown in Figure 1; the derivation of the map is described in detail below.

Bgl II has been shown previously to cut T3 DNA at 44.0% of the genome (5). When Bgl II cut DNA is used as template, a number of high molecular

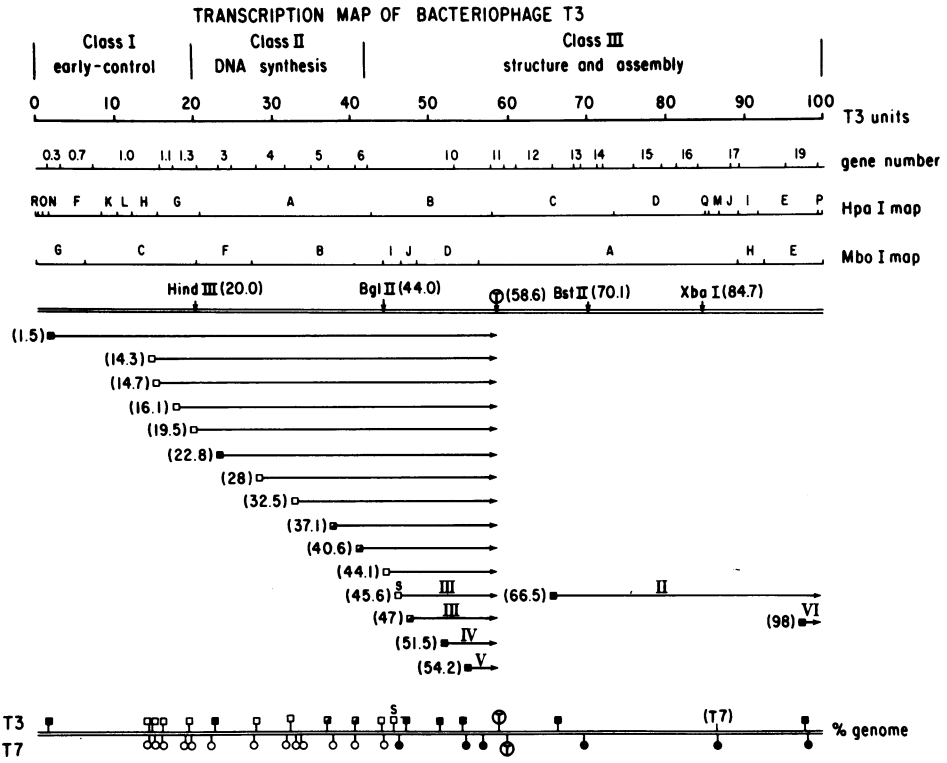


Figure 1. Transcription map of bacteriophage T3. Primary transcripts synthesized in vitro by the T3 RNA polymerase are indicated by arrows. The number in parentheses before each arrow indicates the promoter position (in T3 units). Class III RNAs are also identified by Roman numeral corresponding to the previous nomenclature (14). The termination signal for the T3 RNA polymerase is identified by T. Distances from the left end of the T3 DNA molecule are expressed as percent of genome length [where 1% of the genome equals 1 T3 unit (387 base pairs)]. Positions of the Hpa I, Mbo I, Hind III, Bgl II, Bst E II and Xba I cleavage sites (5) are indicated. At the bottom of the figure the promoter positions for the T3 and T7 RNA polymerases are compared.

■ = T3 strong promoter, salt-resistant
◪ = T3 weak promoter, moderately salt resistant
□ = T3 weak promoter, salt stimulated
□ = T3 weak promoter, salt sensitive
● = T7 salt resistant promoter
0 = T7 salt sensitive promoter

weight bands disappear and a series of lower molecular weight RNAs are detected in the autoradiogram (see Figure 2). From the sizes of these new bands and the knowledge that they represent termination at the Bgl II site, the corresponding promoters may be mapped at (14.3, 14.7), 16.1, 19.5,

22.8, and 32.5%. The existence of two promoters near 14% is confirmed by transcription from a T3 DNA template cut with Hpa I (see below). It should be noted that all of the previously identified class III transcripts (which arise from promoters to the right of 44%) are still present when Bgl II cut DNA is utilized as template.

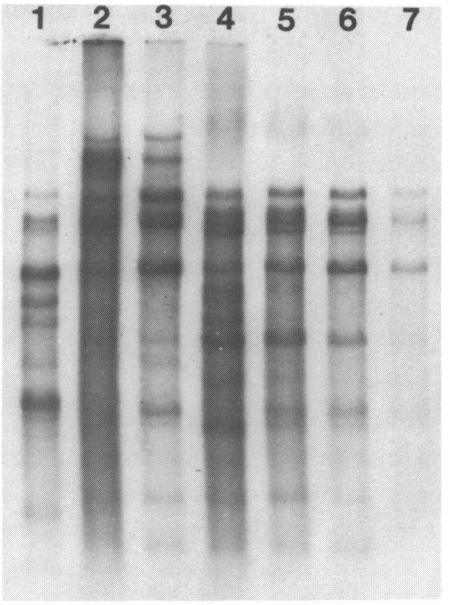
In addition to the class II promoters mapped by transcription of a Bgl II-cut template, three faint RNA bands can be resolved when uncut DNA is utilized as template (Figure 2). These transcripts arise from promoters at 37.1, 40.6, and 44.1 T3 units. The positions of these promoters are further confirmed by analysis of the products transcribed from Hpa I- or Mbo I-cut templates (see below).

The RNA which gives rise to the band identified as 1.5 in Figure 2 is so large that it could only arise from transcription beginning near the left end of the genome. It had previously been shown that the T3 RNA polymerase will transcribe the early region of T3 DNA both *in vitro* and *in vivo* (7,13,28). The existence of a promoter near 1.5 units is confirmed by the detection of appropriately sized transcripts when the template DNA is cut with Bst E II or Xba I prior to transcription (see Figure 3).

In the T7 system, initiation at class II promoters by the T7 RNA polymerase is preferentially inhibited as the salt concentration is increased from 0 to 150 mM KCl (17). This feature of T7 transcription has been useful in discriminating between class II and class III transcripts. Since the T3 RNA polymerase exhibits a similar salt inhibition curve (12,29,30), we anticipated that initiation at the T3 class II promoters might also be salt-sensitive. When Bgl II cut DNA is transcribed in the presence of increasing concentrations of KCl (Figure 2, lanes 4-7), RNAs arising from promoters in the T3 class II region are preferentially inhibited (with the exception of the 22.8 transcript). The preferential inhibition of class II RNA synthesis in the presence of moderate levels of KCl has also been observed by hybridization of the *in vitro* transcripts to Hpa I fragments of T3 DNA (data not shown).

Transcription from templates cut with Hind III, Bst E II, Xba I, Hpa I, and Mbo I.

To refine and confirm the locations assigned to the promoters whose positions were derived from the data in Figure 2, and to extend the analysis to other regions of the genome, template DNA was cut with Hind III, Bst E II, Xba I, Hpa I or Mbo I prior to transcription. RNAs synthesized from each of these cut templates were resolved by electrophoresis in agarose-formaldehyde gels, or by electrophoresis in polyacrylamide gels (Figure 3,


1
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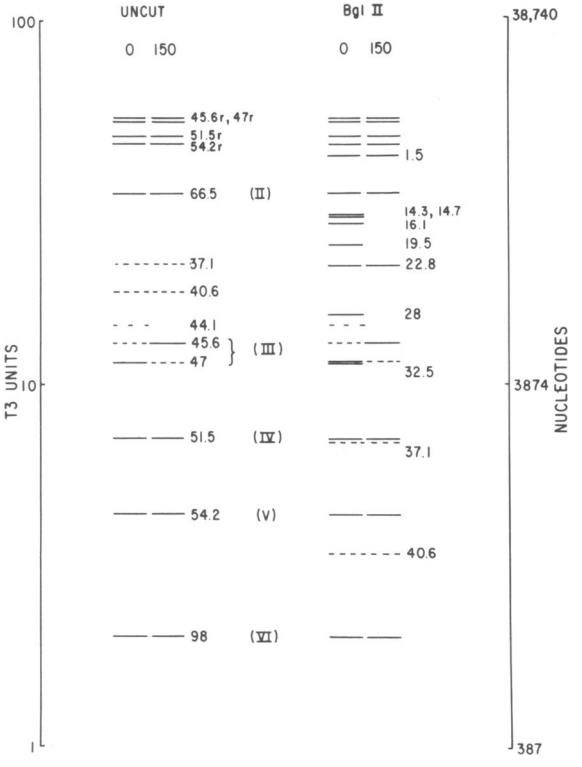
7


UNCUT
0 150
45.6r, 47r
51.5r
54.2r
66.5 (II)
37.1
40.6
44.1
45.6
47 (III)
51.5 (IV)
54.2 (V)
98 (VI)
Bgl II
0 150
1.5
14.3, 14.7
16.1
19.5
22.8
28
32.5
37.1
40.6
100
10
1
T3 UNITS
38,740
3874
387
NUCLEOTIDES

panels A and B). To facilitate the identification of individual RNA species in Figure 3, the transcripts that arise from each promoter position using each cut template are represented diagramatically in panel C. This provides an idealized representation of the autoradiograms in panels A and B. Although many of the predicted RNA species are not resolved (either because they co-migrate with similarly sized transcripts, or because they are too large to be distinguished in this gel system), a well resolved RNA species corresponding to the expected size was observed in at least two instances for each promoter that we have mapped (Table 1). Since the assignment of promoter location is dependent upon the determination of the size of the RNA products, the size of the shortest transcript observed from each promoter was considered to provide the most reliable estimate of promoter location. We estimate that the locations presented in Figure 1 are accurate to within 0.2-1.0 T3 units (80-400 base pairs). Subsequent work will no doubt refine the positions assigned to these promoters, and may uncover additional promoters that we have not detected in this analysis. In this respect, we note that analysis of products transcribed from isolated restriction fragments, which comprise approximately 80% of the genome, supports the positions of the promoters that we have mapped, and does not provide evidence for the existence of additional promoters (data not shown).

The results of these experiments have also permitted us to assign more accurate positions to the class III promoters and to the internal termination signal. For example, Golomb and Chamberlin had previously suggested that the promoters for species IV and V are located near 49% and 52%, respectively (15). When the template is cut with Hpa I prior to transcription, these RNAs are shortened by 0.6 T3 units. From the sizes of the shortened transcripts and the location of the Hpa I site at 58 units (5),

Figure 2. Analysis of T3 RNAs transcribed from uncut or Bgl II cut T3 DNA. Panel A: ^{32}P-labeled RNA was synthesized *in vitro* by the T3 RNA polymerase using uncut T3 DNA (lanes 2 and 3) or Bgl II cut T3 DNA (lanes 4-7) as templates. KCl was present in the reaction mixtures as follows: lane 2, 0 mM; lane 3, 150 mM; lane 4, 0 mM; lane 5, 50 mM; lane 6, 100 mM; lane 7, 150 mM. Lane 1 contains marker RNAs synthesized by the T7 RNA polymerase in the presence of Eco RI cut T7 DNA (17). Electrophoresis was for 6 hours at 55V in a 0.8% agarose-formaldehyde gel. Panel B: The transcripts observed in the autoradiogram are identified by a number indicating the location of the promoter (in T3 units). "-r" indicates that the transcript is a readthrough product resulting from the failure of the polymerase to recognize the termination signal at 58.6% (see text). Class III RNAs are also identified by Roman numerals according to the previous nomenclature (14). Dotted lines represent faint RNA bands.

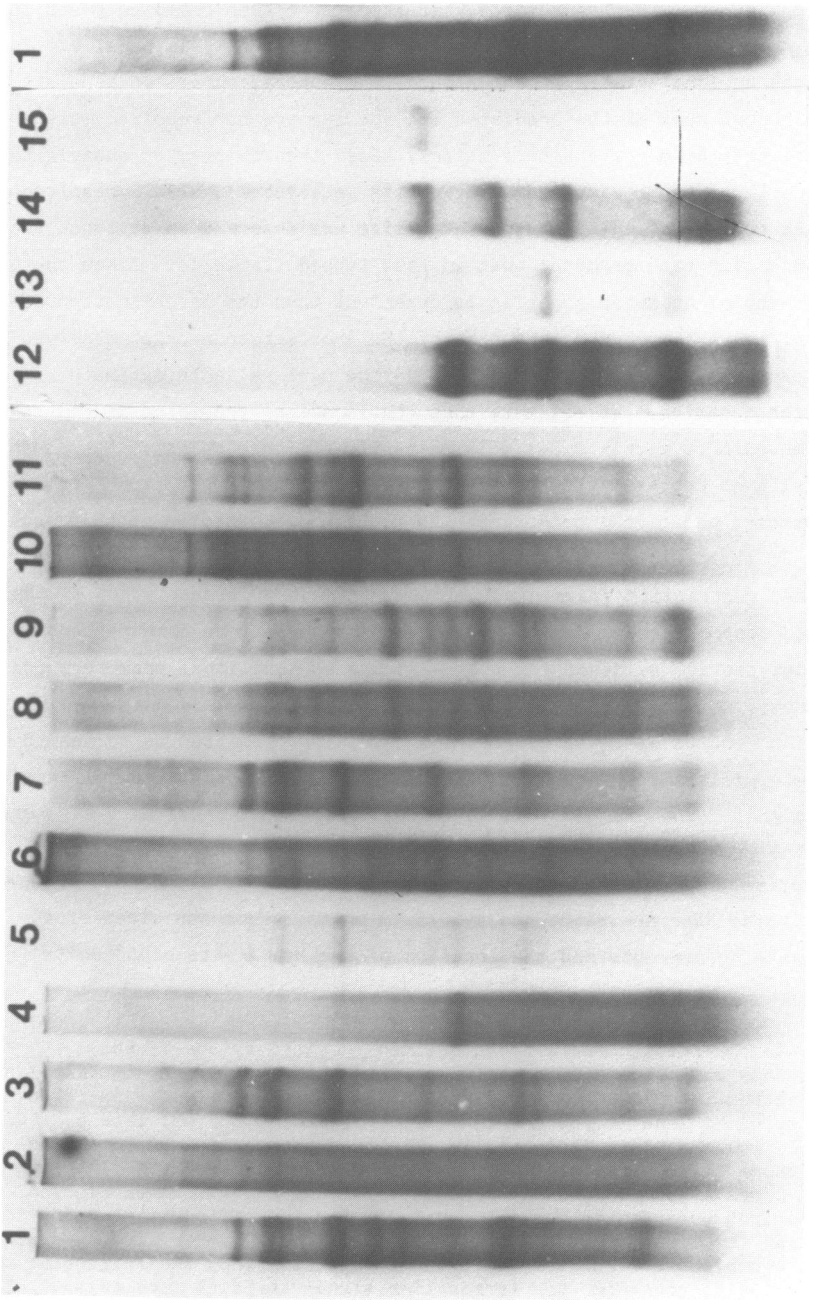


Figure 3a

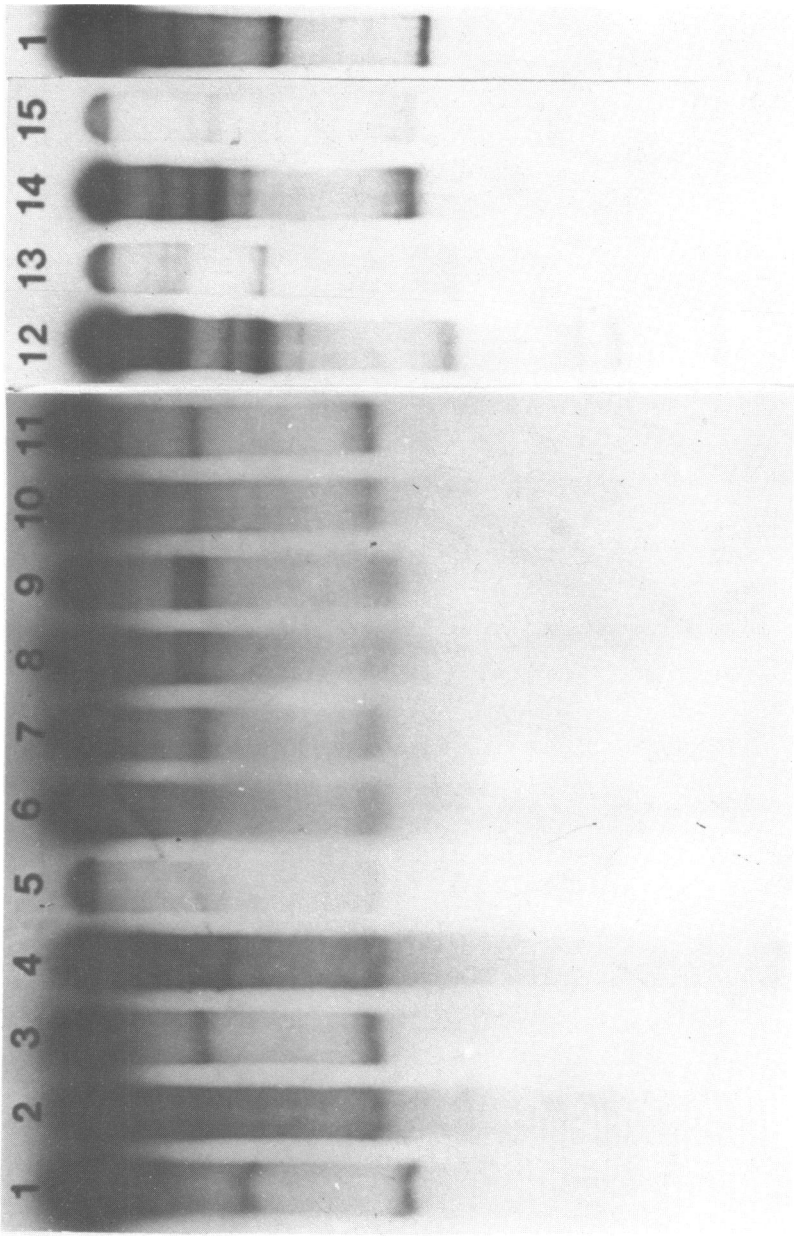
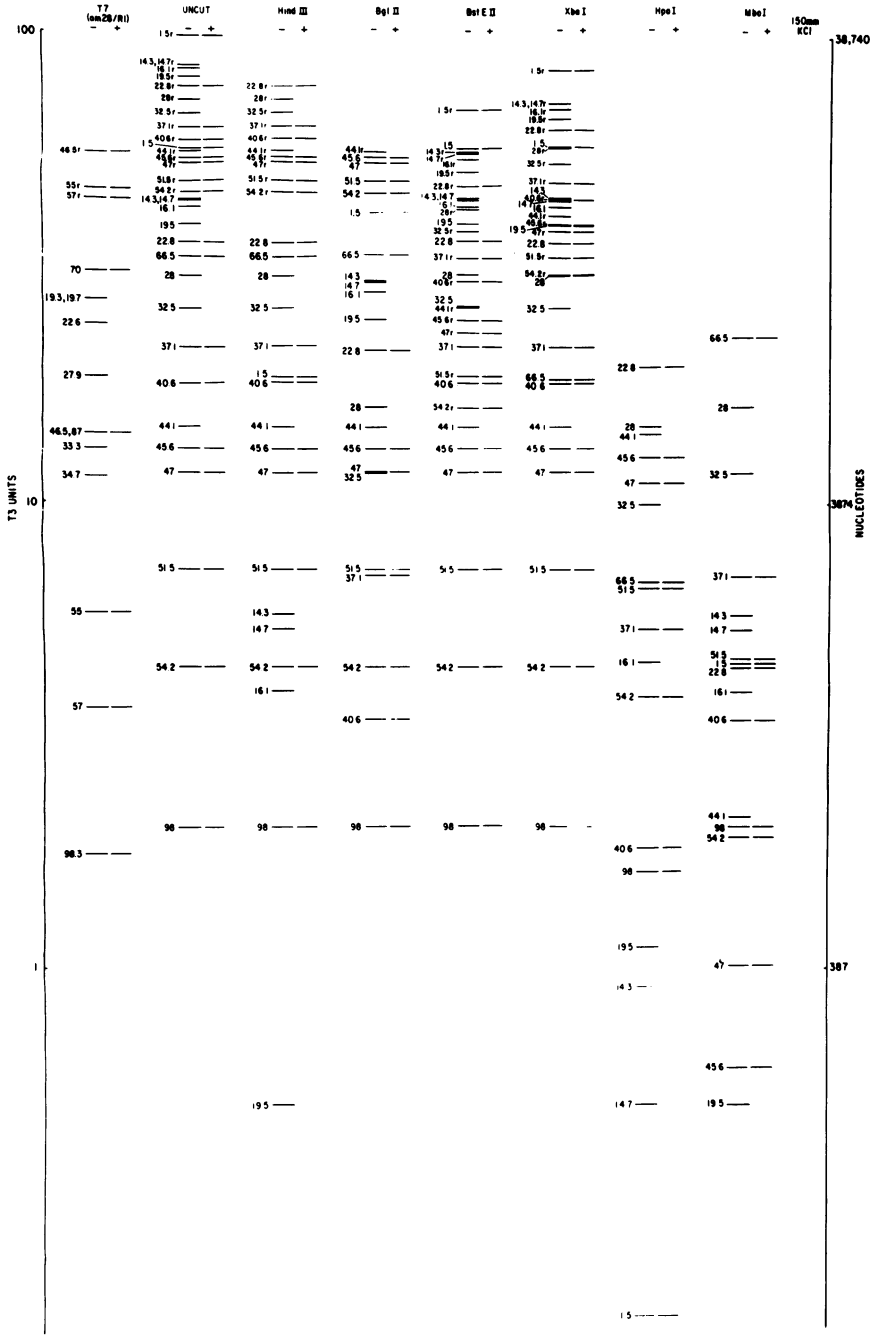


Figure 3b

Figure 3c


T7 (on 28/RI)
UNCUT
Hind III
Bgl II
Bst E II
Xba I
Hpa I
Nde I
150mm KCl
T3 UNITS
NUCLEOTIDES
100
10
1
38,740
3874
387

the promoter locations for these RNA species may be more accurately positioned at 51.5% and 54.2%. Furthermore, it follows that the termination signal must lie 0.6 units to the right of the Hpa I site.

The increased resolution of the gel system employed here has allowed the discrimination of two RNA species previously suggested to arise from a single promoter at approximately 43% (15). From the sizes of the RNAs arising from transcription of Hpa I- or Mbo I-cut templates, the promoter positions for these RNAs have been mapped at 45.6% and 47%.

When Hpa I-cut DNA is employed as a template, species VI RNA (which arises from a promoter at 98%) is shortened by 0.4 T3 unit. Since Hpa I cuts T3 DNA at 99.6% this result confirms the prediction (14) that this RNA species (and probably also the RNA species initiated at 66.5%) is terminated very close to 100% (within 0.1 T3 unit).

In vitro RNA transcripts hybridize to only one strand of T3 DNA.

We have made the assumption throughout this analysis that all transcription is from left to right relative to the map in Figure 1 (14). The effects of cutting the DNA template upon the size of the individual transcripts are consistent with this assumption for all RNA species that we have mapped. To verify that all transcripts arise from the same strand of T3 DNA, and to search for the existence of possible leftward oriented transcripts, the separated single strands of ^{32}P-labeled T3 DNA were isolated and hybridized to unlabeled RNAs that had been transferred to nitrocellulose strips. As shown in Figure 4, all *in vitro* transcripts hybridize to only one strand of viral DNA (the slow strand); this is the same strand transcribed by the T3 RNA polymerase *in vivo* (data not shown). No RNA hybridizing to the fast strand was detected.

Figure 3. Analysis of T3 RNAs transcribed from a variety of cut templates. ^{32}P-labeled RNAs synthesized by the T3 RNA polymerase *in vitro* using T3 template DNA that had been digested with one of several restriction endonucleases were analyzed by electrophoresis in 0.8% agarose-gels in the presence of formaldehyde (panel A) or in 4% polyacrylamide gels (panel B). KCl (150 mM) was present during the transcription reactions shown in lanes 3,5,7,9,11,13 and 15. Template DNAs were as follows: lanes 2 and 3, uncut; lanes 4 and 5, Hind III cut; lanes 6 and 7, Bgl II cut; lanes 8 and 9, Bst E II cut; lanes 10 and 11, Xba I cut; lanes 12 and 13, Hpa I cut, lanes 14 and 15, Mbo I cut. Lane 1 contains marker RNAs synthesized by the T7 RNA polymerase in the presence of Eco RI cut T7 DNA (17).

Panel C is a digarammatic representation of the RNAs expected to arise from each promoter in the presence of a given template. The sizes of the RNA species are indicated in the margins (see Table 1). Each band is identified by a number corresponding to the location of the promoter (in T3 units) which gives rise to the RNA. Readthrough products which result from the failure of the polymerase to consistently recognize the termination signal at 58.6% are designated by the suffix "-r".

TABLE 1. SIZES OF RNAs SYNTHESIZED FROM T3 DNA TEMPLATES CUT WITH DIFFERENT RESTRICTION ENZYMES.

Promoter[2] Location	Salt Resistance[3]	Template[1] Uncut	Hind III	Bgl II	Bst E II	XbaI	HpaI	MboI
1.5	+	57.1[4] (98.5)	18.5	42.5	57.1 (68.6)	57.1 (83.3)	(0.17)[5]	4.5
14.3	0	44.3 (85.7)	5.7	29.7	44.3 (55.8)	44.3 (70.5)	0.9	5.7
14.7	0	43.9 (85.3)	5.3	29.3	43.9 (55.4)	43.9 (70.1)	0.5	5.3
16.1	0	42.5 (83.9)	3.9	27.9	42.5 (54)	42.5 (68.7)	4.5	3.9
19.5	0	39.1 (80.5)	0.5	24.5	39.1 (50.6)	39.1 (65.3)	1.1	0.5
22.8	+	35.8 (77.2)	35.8 (77.2)	21.2	35.8 (47.3)	35.8 (62)	19.6	4.4
28	0	30.6 (72)	30.6 (72)	16	30.6 (42.1)	30.6 (568)	14.4	16
32.5	0	26.1 (67.5)	26.1 (67.5)	11.5	26.1 (37.6)	26.1 (52.3)	9.9	11.5
37.1	(+)	21.5 (62.9)	21.5 (62.9)	6.9	21.5 (33)	21.5 (47.7)	5.3	6.9
40.6	(+)	18 (59.4)	18 (59.4)	3.4	18 (29.5)	18 (44.2)	1.8	3.4
44.1	0_s	14.5 (55.9)	14.5 (55.9)	14.5 (55.9)	14.5 (26)	14.5 (40.7)	13.9	2.1
45.6	$+^s$	13 (54.4)	13 (54.4)	13 (54 4)	13 (24.5)	13 (39.2)	12.4	0 6
47	(+)	11.6 (53)	11.6 (53)	11.6 (53)	11.6 (23.1)	11.6 (37.8)	11	1.0
51 5	+	7.1 (48.5)	7.1 (48.5)	7.1 (48.5)	7.1 (18.6)	7.1 (33.3)	6.5	4.6
54.2	+	4.4 (45.8)	4.4 (45.8)	4.4 (45.8)	4.4 (15 9)	4.4 (30.6)	3.8	1.9
66.5	+	33.5	33.5	33 5	3 5	18.3	6.7	22 5
98	+	2	2	2	2	2	1.6	2

1. Template DNAs were digested with the restriction endonuclease indicated prior to transcription.
2. Position of promoter on the T3 physical map (see Figure 1).
3. Resistance to KCl in reaction mixture: + = highly resistant; 0 = salt sensitive; (+) = moderately resistant (weak) promoter; $+^s$ = salt-stimulated promoter.
4. Size (in T3 units) expected for RNA products that arise from the promoter position indicated. One T3 unit corresponds to 378 nucleotides. Numbers in parentheses indicate the size of the "readthrough product expected from each promoter (see text). Underlining indicates that a well resolved RNA species corresponding in size to that expected has been observed by gel analysis.
5. The RNA is too small to be observed on the gels employed in these experiments.

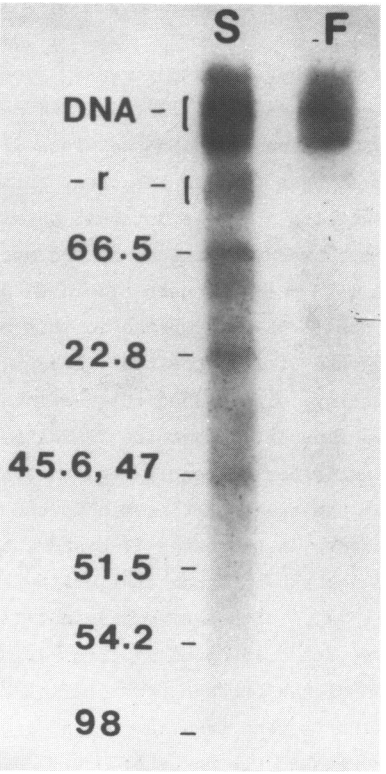


Figure 4. Hybridization of ^{32}P-labeled separated single strands of T3 DNA to T3 RNA transcripts. Unlabeled T3 RNA transcripts were synthesized in vitro from a Bgl II cut DNA template, resolved by electrophoresis in an 0.8% agarose-formaldehyde gel and transferred to nitrocellulose filter strips as described in Materials and Methods. The filter strips were incubated with separated strands of T3 DNA uniformly labeled with ^{32}P, washed, dried, and exposed to X-ray film. The letters F and S at the top of the lanes refer to the fast and the slow strands of the DNA used in each hybridization reaction. Since the template DNA present in the transcription reaction was not removed before electrophoresis, each nitrocellulose strip contains unlabeled DNA which migrates in the region above the largest RNA band (designated DNA in the figure above).

DISCUSSION

We have derived an expanded transcription map of bacteriophage T3. Most of the newly identified transcripts originate from promoters in the region between 15% and 45% of the genome. Previous failure to identify these RNAs can probably be attributed to difficulty in resolving high

molecular weight RNAs in earlier gel systems, and upon the use of reaction conditions that did not favor RNA synthesis from the class II region.

The T3 transcription map shows a strong resemblance to the transcription map of bacteriophage T7 (17,31, see Figure 1). Both viruses have two overlapping transcription units in the class III region of the genome and a large number of promoters in one overlapping unit in the class II region. Both viruses utilize a single internal termination signal near 60%. The major difference between the transcription maps of the two viruses is the absence of a T3 promoter near 87% in T3 DNA (14). Although there is no promoter for the T3 RNA polymerase in this region, there is a promoter for T7 RNA polymerase at this position in T3 DNA [(14); M. Rosa, personal communication; Bailey, unpublished observations]. A further difference between the two viruses is that the T3 RNA polymerase (but not the T7 RNA polymerase) transcribes the early region of the genome.

A striking feature of the transcription map is the predominance of promoters to the left of 58%. In the phage T7 system, the phage RNA polymerase fails to recognize the internal termination signal at 60% about 10% of the time *in vitro* (31). Although we have not directly measured the frequency of termination at the corresponding termination site in T3, the presence of a large number of readthrough products indicates that a similar situation exists in T3. Inefficient termination *in vivo* would decrease the necessity for strong promoters to the right of 58%.

In previous studies a diffuse band that migrated near the origin of polyacrylamide gels was thought to result from an RNA species (species I) that arises from a promoter near 56% (14,15). We have been unable to detect a transcript from this position in any of the reactions that we have analyzed, even though some of these reactions would be expected to give rise to a well-resolved species I RNA. We conclude that there is not a major promoter in the region of 56% as suggested by Chamberlin (14). A similar situation has been shown to exist in bacteriophage T7 (31).

Several investigators have demonstrated that T3 RNA polymerase activity is inhibited by monovalent cation concentrations in excess of 50 mM (12,29,30). However, as previously noted for bacteriophage T7 (17,31), some T3 promoters are more sensitive than others to increasing ionic concentrations. The promoters of the class III region are relatively insensitive to concentrations of KCl up to 150 mM whereas most of the promoters in the class II region are completely inhibited at this KCl concentration (see Figure 2). The exception to this is the class II promoter at 22.8%. The difference in salt sensitivity of promoters is a useful tool in

analyzing the transcripts from various cut templates. Work in our laboratory has demonstrated that the effect of ionic conditions on the selectivity of the bacteriophage T7 RNA polymerase is mediated at the level of initiation, and reflects differences in promoter structure at the class II and class III promoters (17). It will be interesting to determine whether T3 is similar in this respect.

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